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3 **Wnt6 - another player in the Yin and Yang of renal Wnt signalling**

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8 Running title: Role of Wnt6 in diabetic renal fibrosis

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20 Diabetic nephropathy (DN) remains the single most common cause of end-stage kidney  
21 disease, necessitating dialysis or transplantation, in the Western world. Hence, novel  
22 therapies beyond tight blood pressure and glycaemic control are required to slow or reverse  
23 progression of nephropathy in patients with diabetes. Whilst significant efforts have been  
24 made to understand the molecular basis of DN, further delineation of the final common  
25 pathway of renal fibrosis, where the functioning nephrons are replaced by scar tissue, may  
26 identify novel therapeutic targets.

27 The WNT pathway is a highly conserved signalling pathway that is essential during  
28 development in several organs including the kidney. There are 19 mammalian Wnt ligands  
29 and these are spatially regulated during development. During nephrogenesis the secreted  
30 Wnt ligands, Wnt9b and Wnt4 are indispensable and stimulate mesenchymal cells to  
31 differentiate into epithelial cells that subsequently generate the nephron (9). WNT  
32 signalling is carefully regulated by endogenous suppressors of WNT signalling such as  
33 Dickkopf-1 (Dkk1) and Axin. Crosstalk between renal stromal cells and the nephron  
34 epithelia are required to regulate nephron elongation and differentiation including  
35 suppression of Wnt signalling by DKK-1 to allow branching morphogenesis to occur (7)

36 The classical model of Wnt signalling is that Wnt ligands interact with heterodimeric  
37 receptor complexes consisting of a Frizzled (Fz) receptor and low-density lipoprotein-related  
38 receptor 5 or 6 (LRP5/6). Recruitment of axin promotes phosphorylation of the cytoplasmic  
39 tail of the LRP5/6 receptor, which ultimately leads to cessation of B-catenin phosphorylation  
40 followed by its translocation to the nucleus where it binds and activates TCF/LEF family  
41 transcription factors to induce target genes (2).

42 In the normal kidney the Wnt pathway is active in cells in the papilla, however after injury  
43 Wnt pathways become activated throughout the kidney. This activation of Wnt signalling  
44 can be protective or deleterious depending on the cell type. The Wnt pathway has been  
45 implicated in human diabetic renal disease by high throughput transcriptomic analysis and  
46 in preclinical models of diabetic nephropathy and renal injury. Within injured podocytes  
47 there are increased levels of Wnt1, Wnt2b, Wnt4, Wnt6 and Wnt16 (3). In contrast in  
48 mesangial cells, high glucose culture down regulated Wnt4 and Wnt5a expression and  
49 induced apoptosis which was also observed in diabetic rats (4).

50 In this issue, Beaton et al (1) have provided functional insight regarding the role in diabetic  
51 nephropathy of the hitherto poorly characterised Wnt6. As expected Wnt/ $\beta$ -catenin  
52 signalling was increased in the diabetic kidney, however Wnt6 expression was decreased in  
53 the tubulointerstitium of patients with DN. Using preclinical models of DN and renal fibrosis  
54 they found a progressive reduction in Wnt6 expression. They demonstrated for the first  
55 time that during development Wnt6 expression was detectable in the mesonephric duct and  
56 urogenital membrane at E9.5. Wnt6 co-localised with Frizzled 7 (FzD7) expression and  
57 coincided with canonical Wnt signalling in a TCF/Lef reporter mouse. Therefore they  
58 suggest that FzD7 is a putative receptor of Wnt6, for which they provide further evidence by  
59 demonstrating that siRNA knockdown of FzD7 blocked phosphorylation of GSK3 $\beta$  by Wnt6 in  
60 renal tubular cells. This led to their hypothesis that Wnt6 may play a role in epithelial cell  
61 fate. Transfection of renal tubular cells grown in 3D culture with Wnt6 led to new tube-like  
62 protrusions indicating that Wnt6 can drive *de novo* tubulogenesis. In addition, transfection  
63 of renal epithelial cells with Wnt6 prior to or after TGF $\beta$  stimulation prevented epithelial to  
64 mesenchymal trans-differentiation by inhibiting expression of vimentin although this had no  
65 effect on the loss of E-cadherin. Analysis of the promoter revealed that vimentin has a NF-

66 K $\beta$  binding site so the authors explored if non-canonical TGF $\beta$  signalling through NF-K $\beta$  was  
67 involved in the regulation of vimentin. Using TGF $\beta$  stimulation of p65  $-/-$  and IKK $-/-$   
68 fibroblasts they observed that vimentin expression was undetectable compared to wild-type  
69 fibroblasts. This interesting study reveals differential expression patterns of the Wnt ligands  
70 following injury. Loss of Wnt6 is permissive for loss of epithelial integrity and function,  
71 while restoration of Wnt6 may increase repair of the tubular cell population by inducing  
72 tubulogenesis.

73 How do the current findings compare with previous studies examining other Wnt ligands?  
74 During the repair phase following ischemia reperfusion (I/R) injury Wnt2, Wnt2b, Wnt4,  
75 Wnt7b and Wnt10a expression is upregulated (5). Consistent with this, genetic ablation of  
76  $\beta$ -catenin in the renal epithelia has been found to aggravate acute kidney injury (10).  
77 Macrophages may be a major source of Wnt ligands during the repair phase following I/R  
78 injury, with macrophage-derived Wnt7b ligand binding to Fzd4:LRP5/6 on tubular epithelial  
79 cells being critical for the repair phase (5). Wnt7b signalling crosstalk between macrophages  
80 and tubular cells promotes tubular membrane repair and drives epithelial cells through the  
81 G2 arrest as they repopulate the tubules (5). Thus Wnt signalling is critical for kidney repair  
82 following acute kidney injury and inhibition of signalling may be deleterious in this context.

83 Myofibroblasts exhibit increased Wnt/ $\beta$ -catenin signalling following kidney injury. Blockade  
84 of Wnt signalling through systemic administration of DKK-1 inhibits myofibroblast expansion  
85 and renal fibrosis (8). Recent studies by the Humphreys' group have revealed that paracrine  
86 Wnt signalling by the Wnt1 ligand is sufficient to drive fibrosis in the absence of  
87 inflammation (6). Induction of Wnt1 expression specifically in cortical proximal tubular cells  
88 in a transgenic mouse resulted in renal fibrosis by 12 weeks. Although the fibrosis observed

89 was mild there was a significant increase in the number of platelet-derived growth factor- $\beta^+$   
90 and  $\alpha$ -smooth muscle actin<sup>+</sup> proliferating myofibroblasts in the interstitium. Interestingly,  
91 no epithelial cell injury was noted, nor was there evidence of an inflammatory cell infiltrate.  
92 There was, however, a small but significant increase in TGF $\beta$  and Smad3 expression in the  
93 kidneys which indicates cooperative and potentially synergistic convergence of the Wnt and  
94 TGF $\beta$  signalling pathways.

95 These studies demonstrate that there are cell-specific responses to Wnt signalling with  
96 activation being either protective or detrimental to the injured kidney depending on the  
97 context (Figure). While targeting the Wnt signalling pathway represents an attractive novel  
98 anti-fibrotic strategy, further studies will be required to further define the role of specific  
99 Wnt ligands and their receptors to ensure successful translation to the clinic.

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**Figure legend**

**Figure 1:** Dual role of Wnt signalling in kidney injury and repair.

**a)** High glucose results in a decrease in Wnt6, which facilitates increased expression of vimentin , a marker of tubular de-differentiation. **b)** Macrophage derived Wnt7b induces basement membrane repair and tubular epithelial repopulation during the repair phase following ischaemia-reperfusion (I/R) injury. **c)** Over-expression of Wnt1 in cortical epithelial cells is sufficient to drive myofibroblast activation and proliferation in the absence of inflammation.

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